Expert Opinion

Cellular interactions of therapeutically delivered nanoparticles

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Introduction: Nanoparticles (NPs) are used extensively in drug delivery. They are administered through various routes in the host, and their uptake by the cellular environment has been observed in several pathways. After uptake, NPs interact with cells to different extents, depending on their size, shape, surface properties, ligands tagged to the surface and tumor architecture. Complete understanding of such cellular uptake mechanisms and interactions of NPs is important for their effective use in drug delivery.
Areas covered: This article describes the various cellular pathways for NP uptake, and the factors affecting NP uptake and interactions with cells. Understanding these two important aspects will help in the future design of NPs for effective and targeted drug delivery.
Expert opinion: Surface charge and ligands tagged on the surface of NPs play a critical role in their uptake and interaction with cells; so surface modifications of NPs can offer increased drug delivery effectiveness, for example, the coupling of ligands on the surface of NPs can increase cellular binding, and NPs in biological fluids can be coated with proteins and as such can exert biological effects. All of the factors affecting NP uptake need to be investigated thoroughly before interpreting any NP–cellular interactions.

Keywords: cellular interaction, cellular uptake, endocytosis, nanoparticles, targeting

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1. Introduction

Nanoparticles (NPs) are submicrometer-sized particles. Nanoparticles have found widespread applications in the field of drug delivery [1]. The submicrometer size of NPs offers some distinct advantages over microparticles as they are best suited for intravenous delivery [2]. Nanoparticles are used to provide targeted delivery of drugs, to improve bioavailability, to sustain drug gene effect in target tissue, to solubilize drugs for intravascular delivery, and to improve the stability of therapeutic agents against enzymatic degradation [3]. Anatomic complexities of the blood–brain barrier, the branching pathways of the pulmonary system and the tight epithelial junctions of the skin have made it difficult for drugs to reach their therapeutic targets. Nanoparticles can penetrate or overcome these barriers [4]. Nanoparticle-mediated targeted delivery is used to direct NPs to specific tissues [5]. Nanoparticles can be formulated for targeted delivery to the lymphatic system, brain, arterial walls, lungs, liver and spleen or made for long-term systemic circulation [2]. The delivery of drug to target tissue can be achieved in two ways: passive and active. Passive targeting takes advantage of the permeability of tumor tissue. This is also known as the enhanced permeation and retention effect (EPR) [6]. Cell-type specificity introduced by actively targeted NPs through ligand conjugation has been shown to enhance cellular uptake [7]. Depending on the nature of target sites, different

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ligands are used for targeted delivery. Uptake of NPs is proportional to the expression level of receptors on targeted site [8]. Higher concentration/density of ligands on the NP surface leads to higher binding and uptake of NPs by the target site [9].

The targeting capabilities of NPs are influenced by particle size, surface charge, surface modification and hydrophobicity. These parameters can affect cellular uptake, protein binding and translocation from path of entry to the target site [10,11]. Among these, the size and size distributions of NPs are important to determine their interaction with the cell membrane and their penetration across the physiological barriers. The size of NPs for crossing different biological barriers is dependent on tissue, target site and circulation. For the cellular internalization of NPs, surface charge is important in determining whether the NPs would cluster in blood flow or would adhere to, or interact with oppositely charged cells [12]. Nanoparticles administered by various routes come into contact with cells. On contact, NPs entered into different organelles depending on their size. Nanoparticles can enter into cells and the cell nucleus, and can pass the blood–brain barrier. Nanoparticles with different charges have been evaluated for their effect on the host. Anionic NPs have been reported as having no effect on blood–brain integrity, whereas high concentrations of anionic and cationic NPs have been found to be toxic for the blood–brain barriers. Nanoparticles can also pass through loose vasculature of tumor endothelium [7]. Nanoparticles' interactions and uptake also depend on the tumor architecture, targeting ligands and route of administration [7,13,14].

Nanoparticles in the bloodstream also encounter plasma proteins and immune cells. Uptake of NPs by immune cells may occur by various pathways and can be enhanced by adsorption of plasma proteins. This has been assumed to be one of the ways of NP clearance from the site of application. Nanoparticles in a biological medium are involved in drawing several proteins and lipids, resulting in the formation of a 'corona' in slow exchange with the environment [15,16]. Usually, biological identities of NPs undergo changes inside the body. Despite the fantastic potential of NPs in medicine, studies in relation to their cellular interactions are unexplored [5]. Understanding such interactions is important not only for engineering of NPs for cellular uptake, but also for determining toxicity of NPs [5]. Knowledge of NPs' identity and methods to assess them are required. Also, there is a strong need to identify *in vivo* interactions of NPs with biological components. In this article, cellular pathways of NPs' uptake and factors affecting their cellular uptake are reviewed. The interactions of therapeutically used NPs with biological components are also discussed.

2. Pathways for nanoparticle uptake

Nanoparticles are taken up by cellular systems through endocytosis [17]. Endocytosis is a process by which cells absorb molecules from outside by engulfing them with their cell membrane [18]. This process is usually categorized into two phenomena, namely phagocytosis and pinocytosis. Phagocytosis is a cellular phenomenon that describes the process in which phagocytes (specialized cells such as macrophages) destroy foreign particles such as NPs in blood [19]. Transferrin-coated PLGA NPs are highly absorbed by brain endothelial cells and enter cells by means of the caveolae pathway [20]. Similar results have been reported for porous NPs [21]. Gold NPs are usually internalized by a mechanism involving pinocytosis [22]. Pinocytosis occurs by four different mechanisms: macropinocytosis, caveolin-dependent endocytosis, clathrin-dependent endocytosis and clathrin/caveolin-independent endocytosis [23-25]. Macropinocytosis is involved in the formation of lamellipodia-like plasma membrane extensions. Interestingly, NPs > 200 nm can enter cells through macropinosomes [26]. PEGylated-poly-L-lysine NPs have been reported to undergo cellular uptake by macropinocytosis [26]. One of the important endocytic mechanisms is receptor-mediated process. In this process, cellular membrane binds NPs with receptors, wraps around these particles and then pinches off to form vesicles [27]. It is assisted by specific proteins, either clathrin or caveolae [27]. Clathrin coats and spherical caveolae have diameters of 100 – 200 and 50 – 80 nm, respectively [28]. Clathrin-coated pits have the ability to accumulate only NPs up to 100 nm [29]. Uptake of NPs in clathrin-dependent endocytosis is limited to receptor-bound ligands [26]. The internalization of NPs was more efficient for particles smaller than the caveolae. Hence, cellular uptake of 20 – 40 nm NPs was 5 to 10 times more than 100 nm NPs. It is the size of caveolae that restricts internalization of larger particles [30]. Viruses are usually budded at optimal concentration of internalized particles [31,32]. Various studies on targeted drug delivery into cells have shown that the size of particles is indeed an important factor in cellular uptake of NPs. Receptor-mediated endocytosis of NPs is strongly size-dependent, with an optimal radius of 27 – 30 nm for spherical particles [33,34]. The particles near this optimal size are most efficiently taken by receptor-mediated endocytosis [35]. Characteristics such as time, threshold and optimal radii for particle endocytosis are estimated as a function of the binding energy factor, bond elasticity factor, and nonspecific attractive/repulsive factor at the cell–particle interface [36].

Cellular internalization of NPs could happen through any of these pathways depending on their size, shape and nature. The charge on NPs undoubtedly determines the endocytic pathways for cellular entry. Negatively charged NPs are endocytosed by slower rate and are unable to utilize the clathrin-mediated endocytosis pathway. On the other hand, positively charged NPs are internalized rapidly by means of the clathrin-mediated pathway [37]. Endocytosed NPs are usually confined to endosomes. However, endosomal uptake of NPs can be avoided if NPs are delivered by means of liposomes or modifying their surface with cell-penetrating peptides [38].

Nanoparticle size and surface charge as well as the specific ligands attached to the cell surface are crucial players determining NP uptake. Polycaprolactone/PEG/polycaprolactone NPs containing doxorubicin are internalized in tumor cells by endocytosis [39]. Nanoparticles with positively charged groups at their surface such as polyethyleneimine or polyamidoimine dendrimers can induce disruption of plasma membrane. Such disruption in plasma membrane is responsible for nanohole formation [40]. In addition to hole formation, NPs have been found to precipitate at the cell surface. Sometimes, this kind of NP agglomeration at the cell surface results in the disruption of cell function [41]. Smaller NPs interact with cell membranes by forming holes, whereas larger NPs wrap a lipid bilayer around themselves [42].

Modified glycol chitosan NPs have been investigated for cellular uptake mechanism and intracellular fate [43]. Interestingly, these NPs showed an enhanced distribution in the whole cells compared with parent hydrophilic glycol chitosan polymers [43]. *In vitro* experiments with endocytic inhibitors have suggested that several distinct uptake pathways such as clathrin-mediated endocytosis, caveolae-mediated endocytosis and macropinocytosis are involved in the internalization of glycol chitosan NPs. Some glycol chitosan NPs have also been found trapped in the lysosomes on entry [43]. Similarly, poly-L-lysine-PEG-DNA NPs are also taken by several cellular pathways and are trapped in lysosomes on entry [44].

3. Factors affecting uptake and interaction of NPs with cells

Numerous experimental studies have been conducted to probe NP and cell interactions [45]. Nanoparticle uptake and interaction depend on the composition, size, shape, surface charge, passive targeting and surface ligands. Composition of NPs also has been reported to affect their fate inside the body. Nanoparticles are synthesized from different materials. Biodegradable NPs are cleared rapidly from the body [46], whereas non-biodegradable particles accumulate inside the body for extended periods [47].

Nanoparticle uptake by cells is considered to be a two-step process: binding of NPs to the cell surface, followed by uptake of NPs by the specific endocytosis pathway. Nanoparticles have generally higher uptake compared with microparticles. The 100 nm particles have shown greater uptake compared with 1 μm microparticles in the Caco-2 cell line [48]. Particle size has a significant effect on cellular and tissue uptake. In some cell lines, only the submicrometer size particles are taken up efficiently [49]. Nanoparticle uptake by cells is also affected by the shape of the NPs. Shape-dependent influence on the cellular uptake of protein-coated gold NPs has been studied using HeLa cells (ovarian cancer cells), SNB19 cells (brain tumor cells) and STO cells (fibroblast cells). It has been observed that spherical NPs are taken up by cells more efficiently than rod-shaped NPs [50,51]. Shape of NPs affects not only cellular uptake, but also internalization. Spherical particles are internalized at a higher rate than elliptical disks in endothelial cells (**Figure 1**) [52]. Interestingly, polymer carriers of various size (0.1 – 10 μm) have been targeted to intercellular adhesion molecules, and reported similar results [53]. Rod-like particles have shown different endocytosis properties in HeLa cells compared with spheres [54].

Conventional NPs are rapidly cleared from the body by macrophages. To increase the persistence of NPs in the bloodstream, the surface of NPs is modified by hydrophilic polymers such as PEG, polyvinyl alcohol, and so on. Surface modification of NPs has shown potential for medical applications such as drug targeting in terms of cellular binding, internalization and intracellular transportation [55]. PEG is frequently used for the surface modification of various polymeric NPs [56]. This is a hydrophilic, non-ionic polymer and is known to have excellent biocompatibility. The primary interest in preparing PEG-functionalized particles is to improve the long-term systemic circulation of the NPs. In particular, conjugating PEG chains to the surface of proteins or particles has increased the duration in the bloodstream [57]. PEG coating on the surface of PLA has reduced the interaction between the NPs and the enzymes of the digestive fluids. On the other hand, this has increased uptake of encapsulated drug in the bloodstream and lymphatic tissue [56]. PEG has been used successfully for the modification of surface properties of polycaprolactone NPs for the lipophilic drug taxol [58]. The hydrophilic outer shell of mPEG and the hydrophobic inner core of polycaprolactone-taxol have improved the efficiency of loading into mPEG/polycaprolactone NPs. Another good example of PEG-based surface modification of polyhexadecyl cyanoacrylate NPs has also been reported. This has been documented with regard to reducing the natural blood opsonization process of the particles. During this, recognition of NPs by macrophages and the particles' half-life in blood have improved. PEGylated particles showed comparatively higher uptake of drug by the spleen and the brain than conventional non-PEGylated NPs [59].

Besides size of NPs, architecture of tissues and NPs also affects uptake and interactions of NPs with cells. Angiogenic blood vessels in tumor tissues have gaps as large as 600 – 800 nm between adjacent endothelial cells. This loose architecture induces passive targeting, which allows NPs to permeate through these gaps and accumulate inside tumor tissues [60]. Nanoparticles can be localized in tumors by passive targeting [61]. Doxorubicin-loaded polycaprolactone/PEG/polycaprolactone NPs were passively targeted to the tumor tissue by the passive targeting effect. Doxorubicin is released in tumor tissue rather than normal tissue. Doxorubicin in NPs could treat mice bearing subcutaneous C-26 tumors more efficiently [39]. Polyethylene oxide-modified poly(β-aminoester) NPs also showed considerable tumor targeting potential by means of a passive targeting mechanism [62].

Passively targeted NPs suffer from the limitations of specificity. This problem can be overcome by active targeting.

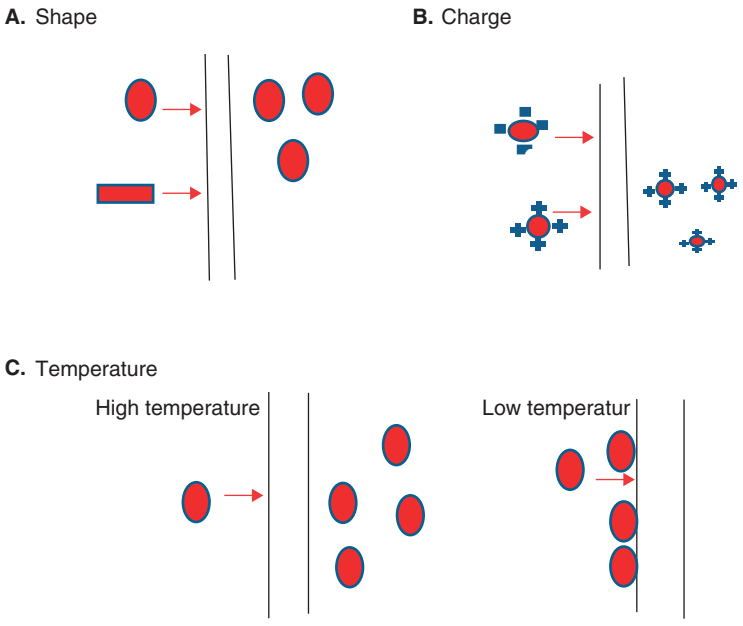

Figure 1. A. Effect of different parameters on cellular uptake of NPs. Spherical particles are efficiently taken up by cells. **B.** Positively charged particles are well taken up by cells. **C.** High temperature allows both cellular uptake and interaction, whereas at low temperature only interaction of NPs takes place.
NPs: Nanoparticles.

Nanoparticles can be targeted to the particulate region of capillary endothelium to concentrate the drug within a particular region and allow it to diffuse from the carrier to the target tissue. Active targeting is usually achieved by attaching NPs to targeting ligand, thereby allowing preferential accumulation of drug in tumor tissue, within individual cancer cells, intracellular organelles or specific molecules. Ligands attached to the surface of NPs also have a role in deciding their fate inside the body. Nanoparticles with specific recognition ligands bound to the surface have good potential for site-selective uptake as well as improved specificity for drug targeting [63]. This strategy has been used to direct NPs to cell surface carbohydrates, receptors and antigens [6]. Ligands attached to the surface can include any molecule that selectively recognizes and binds molecules on target cells [64]. Only those ligands whose antigen or receptors are overexpressed on cancer cells can be used. Antibodies, oligopeptides, carbohydrates, glycolipids and folic acid are the most widely used ligands for targeting different organs and tissues.

Monoclonal antibody-coated poly(lactic-*co*-glycolic) acid NPs used in the co-culture of MCF-10A neoT and Caco-2 cells are localized solely to MCF-10A neoT cells. This has documented cell-specific internalization of NPs [65]; but use of monoclonal antibodies is associated with certain disadvantages. Monoclonal antibodies show poor *in vivo* mobility and reduced uptake owing to their larger sizes. Moreover, monoclonal antibodies raised from murine and chimeric mouse for humans are immunogenic and as such produce harmful side effects. In sharp contrast, peptides have low immunogenicity; but peptides show increased diffusion rates in tissues owing to their smaller size [66,67]. Hence, use of peptides is considered to be a better alternative approach to monoclonal antibodies. Receptors for peptides are highly expressed on a variety of neoplastic and non-neoplastic cells [68]. Receptor-targeting peptides undergo internalization by means of receptor-mediated endocytosis. Attachment of a positively charged tat peptide to the NPs' surface has resulted in efficient internalization of NPs through electrostatic interactions. Such an attachment of NPs with tat peptide has increased further the NPs' clearance from the vascular compartment [69]. Functionalization of gold NPs with tat protein has allowed penetration across the cell membrane and entry into the nucleus [70]. Gastrin-releasing peptide receptors are overexpressed in various human tumors such as glioblastoma, small cell lung, gastric, pancreatic, prostrate, breast, cervical and colon cancer [71-74]. Gold NPs conjugated with bombesin peptide can penetrate cells that are overexpressing gastrin-releasing peptide receptors [68]. Peptidomimetic NPs can provide stronger interaction with surface receptors on tumor cells, resulting in higher uptake and reduced drug resistance. Self-assembled NPs conjugated with peptidomimetic antigens to dendritic cells have resulted in subsequent activation of T cells. The activation of T cells has mediated adaptive immune response [75].

Tumor cells express multiple receptors. These receptors can be targeted for increasing the efficacy, selectivity and

specificity of anticancer drugs. Estrogen receptor-positive MCF-7 breast cancer cells have been used to examine the efficiency of 100 – 300 nm tamoxifen-loaded polycaprolactone NPs. The location of estrogen receptor on the periphery of the nuclear membrane has increased the therapeutic benefit of tamoxifen-loaded NPs [76]. Similarly, tamixifen gold NP conjugates selectively targeted estrogen receptor-α in human breast cancer cells with up to 2.7 times enhanced potency. It has been found that plasma membrane-localized estrogen receptor-α facilitates selective endocytotic transport of these and other therapeutic NP conjugates [77].

Transferrin is another well-studied ligand for targeted delivery to tumor cells. Transferrin receptors are also expressed on the surface of tumor cells. Transferrin ligand is actively taken up by transferrin receptors by means of receptor-mediated endocytosis. Gold NPs conjugated to transferrin ligand have been internalized into nasopharyngeal carcinoma cells. This has resulted in localization of most of the NPs in the cytoplasm of cells [78]. Some applications require the binding of NPs to specific protein or polysaccharide ligands on the cell surface. Such binding is also affected by particle size. An increase in particle size from 50 to 200 nm reduces the affinity of biotin–streptavidin interactions; whereas higher concentration of ligands on particle surfaces shows stronger binding with their counterparts [9].

A small non-antigenic molecule folic acid can also be used for targeted delivery [79]. Folic acid has some advantages over transferrin or antibodies as a ligand for long-circulating carriers. This is due to a much smaller molecule, unlikely to interact with opsonins, and is coupled easily to a PEG chain without loss of receptor binding activity [14]. Folic acid is a stable, inexpensive, non-immunogenic molecule. It has very high affinity for its cell surface receptors. Folate receptors are considered to be useful targets for tumor-specific drug delivery. Folate receptors have been observed to be upregulated in many human cancers, including ovary, brain, kidney, breast and lung [80]. Further, folate density increases with the increase in cancer stage [81]. Keeping these in view, folic acid has been conjugated to NPs for targeting folate receptors on tumor cells [8]. Uptake of folate-conjugated NPs has been found to be proportional to folate expression level in tumor cells [8]. Targeting tumors with folate-targeted nanocarriers is now considered to be a popular approach [82]. Folic acid conjugated to mesoporous silica NPs has shown five times more internalization by cancer cells that are expressing folate receptors at higher levels [83]. Similar results have been reported for paclitaxel-loaded folate-conjugated PEG/polycaprolactone micelles. PEG/polycaprolactone micelles were endocytosed into MCF-7 cells through interactions with overexpressed folate receptors [84]. Surface-modified PEG-polycaprolactone particles with folate, on loading with paclitaxel, have shown increased cytotoxicity [85]. Cell uptake and tumor retention are significantly enhanced by folate-coated gadolinium NPs. Thus, the benefit of folate ligand coating is to facilitate tumor cell internalization and retention of gadolinium NPs in the tumor tissue [86]. Doxorubicin-loaded folate-PEG-functionalized gold NPs have been used for targeted delivery to folate receptor-positive cells. The doxorubicin nanocarriers show higher cytotoxic effect on folate receptor-positive cells (KB cells) than folate receptor-negative cells (A549 cells) [87]. Folate-PEG-grafted gold NPs have been selectively taken by KB cells that are expressing folate receptors [80]. Interestingly, folate-targeted liposomes show rapid accumulation in IGROV-1 tumors [88]. Protoporphyrin-IX-loaded folic acid-conjugated chitosan NPs are internalized by HT29 and Caco-2-cell lines by means of receptor-mediated endocytosis [89]. For these reasons, NPs with high affinity for folate receptors are now in development.

Wheat-germ agglutinin-conjugated poly(lactic-*co*-glycolic) acid NPs containing isopropyl myristate have shown a stronger cell killing effect. This was owing to more efficient cellular uptake by means of wheat-germ agglutinin receptor-mediated endocytosis in A549 and H1299 cells [90]. Carbohydrates on the cell surface contribute a variety of communications between the cell and its environment. The 2-methacryloyoxyethyl phosphorylcholine NPs bearing hydrazide groups on reaction with levulinoyl mannosamine-treated HeLa cells have shown NP accumulation. This indicates that hydrazide groups of the NPs have reacted with ketone groups of carbohydrates on the surface of HeLa cells. The NPs also recognized levulinoyl mannosamine-treated HeLa cells because of the ketone-functionalized unnatural carbohydrates on their surfaces [91]. Lectin carbohydrate is a classical example of active drug targeting. Lectins are able to detect changes on cell surface carbohydrates of tumor cells. This interaction can be made use of by direct lectin targeting or reverse lectin targeting of drug molecules. Unfortunately, the drug delivery systems based on this strategy were found to deliver the drug molecule to whole organs, and therefore to be harmful to other tissues [92].

Charge on NPs can have an influence on uptake and interaction with cells; whereas neutral functional groups are excellent at preventing interactions of unwanted NPs with cells. Most charged functional groups are responsible for active NP interactions with cells [93]. Cho *et al.* have recently examined the role of surface charge in internalization of gold NPs [94]. Similarly, the uptake of iron oxide NPs functionalized with differently charged carbohydrates has been studied in human cervical carcinoma cell lines [95]. In particular, cationic and anionic NPs have been observed to follow different internalization pathways to enter cells. Internalization of negatively charged NPs was believed to occur through nonspecific binding and clustering of particles on cationic sites of the plasma membrane and their subsequent endocytosis. Cationic particles are well known to bind to negatively charged groups on the cell surface (**Figure 1**) [96]. Interestingly, positive charge has an enhancing effect on the uptake rate compared with neutral

or negatively charge carriers [96]. Brain uptake rates of anionic NPs have been reported to be superior to cationic and neutral NPs [97]. Negatively charged NPs have displayed a less efficient rate of endocytosis. However, positively charged NPs are internalized rapidly by means of a clathrin-mediated pathway. The slight changes in surface functionalities of NPs bearing cationic surface groups can lead to varying amounts of cellular internalization [98]. This has indicated that the effect of NPs' surface properties on their interaction with cells is far more complicated than understood at present. Similarly, polyethyleneimine coating on mesoporous silica NPs has enhanced cellular uptake of siRNA and DNA constructs. Furthermore, positive charge of polyethyleneimine-coated NPs has led to strong electrostatic interactions with negatively charged cell membrane [99]. Positively charged NPs depolarize the plasma membrane, leading to membrane potential perturbations and increased Ca^{2+} influx (**Figure 2**). This has inhibited the proliferation of normal cells, whereas malignant cells remain unaffected [5]. Different surface charges on NPs have resulted in repulsion, adhesion, or penetration into lipid bilayers. The binding between NPs and lipid bilayers is mainly dictated by electrostatic interactions between functionalized ligands of NPs and lipid bilayer head groups. On penetration of NPs into lipid membrane, the NPs associated with membrane disruption. The level of penetration and membrane disruption increased with increasing charge density on NPs [100].

Charged NPs can also induce hemolysis, thrombogenicity and activation of complement system. Hemolytic tendency also increases with an increase in charged groups [101]. Hydrophilic surfaces of NPs also induce hemolysis [102]; whereas the addition of PEG to the NPs' surface has been shown to reduce hemolytic activity [103]. Nanoparticles have also been reported to induce activation and platelet aggregation. Decorating particle surfaces with PEG decreases platelet aggregation and activation [104]. Nanoparticles' surface charge has also caused activation of complement system. Charged particles have more actively invoked complement system than neutral ones [105,106]. Another study recently reported that complement system activation by positively charged NPs was suppressed by phospholipid and BSA loading in the NPs [107].

The biological activity and biokinetics of NPs depend on different parameters, such as size, shape, chemistry, crystallinity, surface properties (area, porosity, charge, surface modifications, coating), agglomeration state, biopersistence and dose. These parameters are likely to modify biological responses, such as translocation across epithelia to other organs, induction of oxidative stress, binding to proteins and receptors, and localization in cellular organelles as mitochondria. As NPs today are more often used in different products, there is an increased risk of exposure of workers, consumers and the general public. Exposure to NPs could be through the use of consumer products, emerging biomedical applications of NPs as drug-delivery agents, biosensors, or imaging contrast agents that involve deliberate, direct ingestion or injection of NPs into the body. Nanoparticles are administered in several ways, such as oral, intravenous, cutaneous, intraperitoneal, and so on [108,109]. After administration into the body, NPs interact with biological components such as proteins and cells. Thereafter, they are distributed to different organs of the body. There is a growing body of literature that details various degrees of adverse biological effects induced by NPs at cellular, subcellular and molecular scales [110-114]. At present, no standard protocol is available for nanotoxicity testing. However, the key elements for toxicity screening strategy should include physicochemical characterization of NPs, *in vitro* assays (cellular and non-cellular) and *in vivo* studies. Further, it is important that the toxicity testing design should be pragmatic and mechanism-based to draw final conclusions about toxicity of NPs.

4. Expert opinion

Cellular uptake and interaction of NPs are affected by the size, shape, surface charge, tumor architecture and the ligand tagged to the surface of the NPs. The composition and surface properties of NPs play a crucial role in their interaction with cells. Nanoparticles are synthesized using different kinds of material. Biodegradable materials are degraded but non-biodegradable materials are retained inside the body for a longer time. Hence, non-biodegradable particles will interact with cellular components for a longer time. Most NPs are internalized through endocytosis and remain trapped in endolysosomal vesicles. Nanoparticles with neutral surface coatings resist the interaction with cells and consequently display minimal internalization. Studies have also reported the uptake of negatively charged NPs into cells, despite their repulsion by negatively charged membrane. Positively charged NPs, however, are most effective in crossing cell-membrane barriers and localizing in the cytosol by depolarizing the membrane to a greater extent compared with other particles. Cellular uptake and interactions of NPs can be modulated by changing the surface properties and varying the nature of the ligands attached to them. Nanoparticle interactions with cells depend not only on slight structural changes in the surface ligands but also on the spatial arrangement of ligands on the NPs' surface. For targeted delivery, ligands are conjugated to the surface of NPs. Conjugation techniques are standardized in order to maintain the activity and specificity of ligands.

For drug delivery, cellular targeting of NPs is very important. Nanoparticles generally end in endosomes or lysosomes followed by degradation. Chemical properties such as surface charge may also determine the fate of NPs in cells. Surface modifications of NPs offer ways for cellular

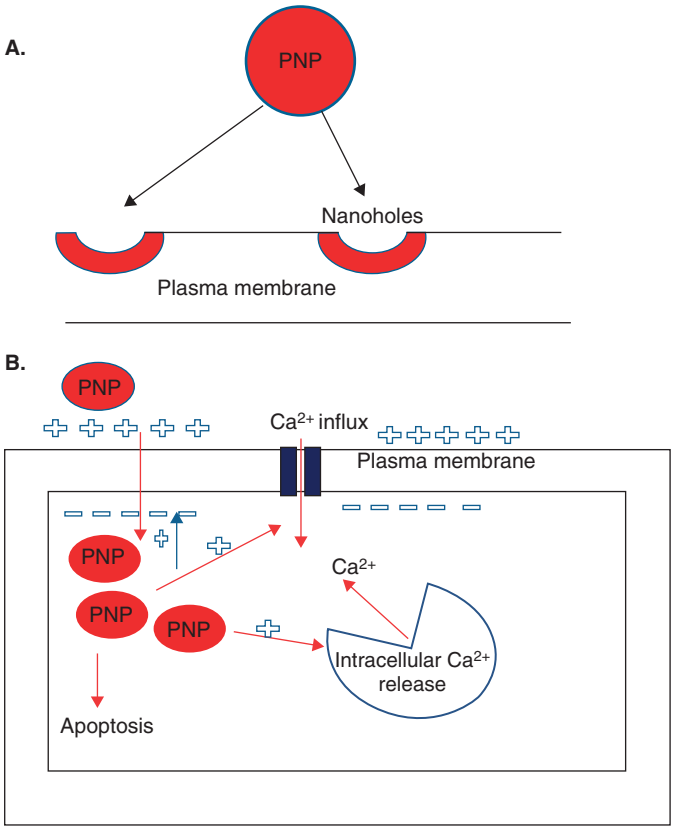

Figure 2. A. Mechanisms of interaction of positively charged PNPs with plasma membrane. PNPs cause nanohole formation in plasma membrane. **B.** Uptake of PNP causes depolarization of membrane potential, increases influx of Ca^{2+} ions and induces release of intracellular calcium stores.
PNP: Positively charged nanoparticle.

binding, uptake and intracellular transport. Coupling of ligands on the surface of NPs also increases cellular binding. Nanoparticles in biological fluids can be coated with proteins and as such can have biological effects. Therefore, more extensive studies are needed to investigate binding affinities and stoichiometries for different protein NP formulations. All the factors affecting NP uptake need to be investigated thoroughly before interpreting any NP–cellular interactions. This is just the beginning towards investigating NP–cell interactions; more extensive studies are needed to reach the final conclusion.

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Declaration of interest

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